DR ANTOINE RAUCH (Orcid ID : 0000-0002-1182-4131)
DR FANNY LASSALLE (Orcid ID : 0000-0003-0494-823X)
MRS SOPHIE SUSEN (Orcid ID : 0000-0001-5953-163X)

Article type : Original Article

Coagulation biomarkers are independent predictors of increased oxygen requirements in COVID-19

Antoine Rauch[*], Julien Labreuche[†], Fanny Lassalle[*], Julien Goutay[‡], Morgan Caplan[‡], Leslie Charbonnier[§], Aurelien Rohn[§], Emmanuelle Jeanpierre[*], Annabelle Dupont[*], Alain Duhamel[†], Karine Faure[¶], Marc Lambert[**], Eric Kipnis[††], Delphine Garrigue[§], Peter J. Lenting[§§], Julien Poissy[‡‡], Sophie Susen[*]

* Univ. Lille, Inserm, CHU Lille, Department of Hematology and Transfusion, Pôle de Biologie Pathologie Génétique, Institut Pasteur de Lille, UMR1011-EGID, 59000, Lille, France

† Univ. Lille, CHU Lille, ULR 2694 - METRICS : Évaluation des technologies de santé et des pratiques médicales, F-59000 Lille, France

‡ Centre Hospitalier Universitaire Lille, Intensive Care Department, Pôle de Réanimation, F-59000 Lille, France

§ Centre Hospitalier Universitaire Lille, Emergency Department, Pôle de l'Urgence, F-59000 Lille, France

¶ Univ. Lille, Inserm, CHU Lille, Department of Infectious Diseases, Institut Pasteur de Lille, UMR1019-CIIL, 59000, Lille, France

** Univ. Lille, Inserm, CHU Lille, Department of Internal Medicine, INSERM U 1167, Institut Pasteur, 59000 Lille, France

†† Univ. Lille, CNRS, Inserm, CHU Lille, Surgical Critical Care, Department of Anesthesiology and Critical Care, Institut Pasteur de Lille, U1019 - UMR 9017 - CIIL - Center for Infection and Immunity of Lille, F-59000 Lille, France

‡‡ Univ. Lille, Inserm U1285, CHU Lille, Pôle de réanimation, CNRS, UMR 8576 - UGSF - Unité de Glycobiologie Structurale et Fonctionnelle, F-59000, Lille, France

§§ Inserm, UMR_1176, Université Paris-Saclay, 94276 Le Kremlin-Bicêtre, France

Corresponding author:

Julien Poissy, julien.poissy@chru-lille.fr, Pôle de Réanimation, Hôpital Roger Salengro, Rue Emile Laine, CHU Lille, 59037 Lille cedex, France.

Please cite this article as doi: 10.1111/JTH.15067

Phone +33320444084 Fax +33320446504
&
Sophie Susen, sophiesusen@aol.com, Institut Cœur-Poumon (Heart and Lung Institute), Hemostasis Department, Bd du Pr Leclercq, CHU Lille, 59037 Lille cedex, France.
Phone +33320444891, Fax +33320446504

Running title: Coagulation and Oxygen Requirement in COVID-19

Disclosures: No disclosures relevant to the manuscript

Essentials:

- VWF levels are associated with severity and oxygen need in COVID-19 at admission
- Low FVIII/VWF ratio at admission is predictive of increased oxygen requirements
- Coagulation biomarkers predict outcome independently of major comorbidities in COVID-19
- FVIII is predictive of early thrombotic events irrespective of BMI in COVID-19

Acknowledgments:
The authors wish to thank Laureline Bourgeois, Aurélie Jospin, Catherine Marichez, Vincent Dalibard, Bénédicte Pradines, Sandrine Vanderziepe, all the biologists and technicians of the Hemostasis Department and the Lille COVID Research Network (LICORNE) for their support during the COVID-19 pandemic.

Data Sharing: For original data, please contact sophiesusen@aol.com

Funding: This study was supported by the French government through the Programme Investissement d'Avenir (I-SITE ULNE / ANR-16-IDEX-0004 ULNE) managed by the Agence Nationale de la Recherche.

Abstract word count: 238
Text word count: 4207
Tables: 3
Figures: 2
Number of References: 43
Supplemental Material: 4 Figures and 1 Table

ABSTRACT

Background: Hypercoagulability seems to contribute to SARS-CoV-2 pneumonia pathogenesis. However, age and metabolic syndrome are potential cofounders when assessing the value of coagulation biomarkers prediction of COVID-19 outcomes. We assessed whether coagulation biomarkers, including FVIII and VWF levels, measured at time of admission were predictive of COVID-19 adverse outcomes irrespective of age and major comorbidities associated with metabolic syndrome.

Methods: Blood was sampled at admission in 243 adult COVID-19 patients for analysis of coagulation biomarkers including FVIII and VWF on platelet-poor plasma. The association between baseline CRP, aPTT ratio, PT ratio, D-dimers, fibrinogen, FVIII, VWF:Ag and FVIII/VWF:Ag ratio levels and adverse outcomes (increased oxygen requirements, thrombosis and death at day-30) was assessed by regression analysis after adjustment on age, sex, body mass index, diabetes and hypertension.

Results: In univariable regression analysis increased CRP (SHR, 1.68; 95%CI, 1.26 to 2.23), increased fibrinogen (SHR, 1.32; 95% CI, 1.04 to 1.68) and decreased FVIII/VWF:Ag ratio (SHR, 0.70 ; 95% CI, 0.52-0.96) levels at admission were significantly associated with the risk of increased oxygen requirement during follow-up**.** Leucocytes (SHR, 1.36; 95%CI, 1.04 to 1.76), platelets (SHR,1.71; 95%CI, 1.11 to 2.62), D-dimers (SHR, 2.48; 95%CI, 1.66 to 3.78), FVIII (SHR, 1.78; 95%CI, 1.17 to 2.68) were associated with early onset of thrombosis after admission. After adjustment for age, sex, BMI, hypertension and diabetes, these associations were not modified.

Conclusion: Coagulation biomarkers are early and independent predictors of increased oxygen requirement in COVID-19 patients.

Keywords: SARS-CoV2, Oxygen, Factor VIII, von Willebrand Factor, Body Mass Index

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is frequently associated with laboratory markers of hypercoagulability [1]. These coagulation changes, mainly characterized by increased D-dimers and fibrinogen levels, are generally observed in critically ill patients, especially those with hypoxemia reflecting inflammation [2]. This increase in D-dimers at admission, without signs of disseminated intravascular coagulation, has been reported to be associated with the risk of death [3,4] .

Evidence for an increased risk of thrombosis in COVID-19 has been first identified through clinical manifestations of large vessels thrombosis. Venous thromboembolic events (VTE) and pulmonary embolism (PE) are reported in 20–30% of Intensive Care Unit (ICU)-patients [5–8] and other thrombotic complications, such as arterial thrombosis or thrombosis of central lines and in extracorporeal circuits have also been described [6]. All post-mortem analyses have confirmed the high frequency of pulmonary vascular thrombosis in ventilated and non-ventilated patients receiving thromboprophylaxis or not [9,10]. Post-mortem histological data also show local direct vascular injury characterized by severe endothelial injury with intracellular SARS-CoV-2, widespread vascular thrombosis with microangiopathy and occlusion of alveolar capillaries, and angiogenesis [11,12]. Altogether, these findings suggest that COVID-19-induced hypercoagulability and inflammation result in both microangiopathy involved in multiple organ failure and macroangiopathy involved in large vessels thrombosis [13].

An increased risk for more severe forms of COVID-19 with ICU admission and death is associated with age, sex, body weight, hypertension and diabetes [14–16]. All these factors increase the risk for thrombotic disease in part through the generation of hypercoagulation. Von Willebrand factor (VWF) and factor VIII (FVIII) are also elevated in patients with COVID-19 [6,7,17,18]. FVIII and VWF are associated with inflammation and thrombotic risk but are also related to endothelial damage. Lung endothelium is a dominant source of circulating VWF, the levels of which might be differentially altered compared to FVIII that is mainly synthetized in the liver[19]. Investigations as to whether VWF and FVIII levels are related to outcome in COVID-19 disease have not been reported yet. Whether the laboratory markers of hypercoagulability, including FVIII and VWF, and the risk of thrombosis in COVID-19 are mainly explained by comorbidities or whether they independently reflect SARS-CoV-2 induced vascular damage has not yet been elucidated.

The objective of the study was to assess whether hypercoagulability markers in COVID-19 patients, including FVIII and VWF levels, measured at admission to the emergency department were predictive of increased oxygen requirement and to evaluate the influence of major comorbidities, including age, sex, body weight, diabetes and hypertension on this relation. We also aimed to evaluate if these hypercoagulability markers were predictive of thrombotic events.

PATIENTS AND METHODS

Patients

Consecutive adult patients admitted for COVID-19 infection were recruited from the emergency department (ED) of the Lille University Hospital between March 20th and April 17th 2020. Inclusion criteria were : individuals aged 18 years or older with either a positive COVID-19 nasal or tracheal real time - reverse transcriptase – polymerase chain reaction (RT-PCR) or with radiological signs of interstitial pneumonia on chest x-rays or computed tomography (CT) scan and a high probability score according to the score for COVID-19 of Liao et al[20]. Following admission, all hospitalized patients received thromboprophylaxis as standard of care unless contra-indicated. Patients admitted while treated with direct oral anticoagulant or vitamin K antagonists were switched to curative heparin therapy. Ward patients received thromboprophylaxis with enoxaparin 4000 IU or 6000 IU once daily according to their body weight. ICU patients received enoxaparin or unfractionated heparin according to their renal status and the need for invasive procedures. In overweight and obese patients, the dosing regimen was adapted according to the ESC proposals [21]. From April 1st onwards, patients received thromboprophylaxis according to the GFHT/GIHP proposals [22]. Limitation, witholding or withdrawal of life-sustaining treatment in the ICU was based on French guidelines in compliance with French law [23]. Deaths in this context were recorded to analyze the real contribution to death of SARS-CoV-2 infection.

Data collection

Epidemiological data, demographic, past medical history and treatments, clinical data and outcomes were prospectively collected from the hospital electronic medical records from emergency department admission to hospital discharge. Comorbidities including hypertension and diabetes were defined according to the presence of an antihypertensive or antidiabetic drug at baseline or according to the medical records. Body mass index was measured upon admission. The clinical status of patients who were directly discharged home from the emergency department was assessed at day 30 by phone interview. The study was approved by the Institutional Review Board (N°CPP 20-LILL-02, NCT04341792) in strict compliance with the French reference methodology MR-004 and informed consent was obtained from all participants.

Laboratory testing

For each subject, a 3 mL blood sample was collected at admission on a 0.109M trisodium citrate tube (BD Vacutainer®, BD Diagnostics, Franklin Lakes, NJ). All haemostasis tests were performed on platelet poor plasma obtained after double centrifugation of citrate tubes at 2000g for 15 minutes at room temperature. Assays included prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, D-dimer, FVIII and VWF antigen (VWF:Ag) levels. The PT, APTT and fibrinogen assays were measured on a STA R Max® analyser (Diagnostica Stago SAS, Asnières sur Seine, France) using STA Neoplastin R®, Triniclot® APTT HS, T Coag® and STA Liquid Fib® (Diagnostica Stago SAS, Asnières sur Seine, France). D-dimers levels were measured in µg/ml Fibrinogen Equivalent Units (FEU) using an immunoturbidimetric latex-particle assay Liatest DDI-Plus® on the STA R Max analyser (Diagnostica

Stago SAS, Asnières sur Seine, France). The reading area is equal to the measuring area and range from 0.27µg/mL (FEU) to 20 µg/mL (FEU). FVIII activity was measured by a one-stage clotting APTT-based assay using Triniclot® APTT HS, T Coag® and factor VIII-deficient plasma, on a Sysmex CS 2400 analyser (Siemens Healthineers AG, Erlangen, Germany). VWF:Ag was measured using an immunoturbidimetric assay, LIAPHEN vWF:Ag (HYPHEN BioMed, Neuville-sur-Oise, France). Other lab tests including a complete blood count, C-reactive protein (CRP), high sensitivity cardiac troponin, lactate dehydrogenase (LDH) levels and ABO blood group were prospectively assessed by standard methods as part of patient's care in the Biology and Pathology Center (CHU Lille).

Study outcomes

The primary outcome of the study was the increase in oxygen requirement defined as a need for re-admission after discharge for a limitation of activities and/or a change in oxygen requirements (no oxygen, supplemental oxygen, non-invasive ventilation or high-flow oxygen, invasive mechanical ventilation or ECMO) and/or death related to acute respiratory distress syndrome without limitation, witholding or withdrawal of life-sustaining treatment at day 30. This composite criterion includes any worsening of the respiratory status in patient and comprises different severity of illness without focusing on the most critically-ill patients. The secondary outcomes were occurrence of any thromboembolic event (symptomatic PE, deep vein thrombosis (DVT), catheter-related thrombosis, myocardial infarction (MI) or stroke) at day 30 and all-cause mortality at day 30. The association between outcomes and the baseline values of Leucocytes, lymphocytes, monocytes, platelets, CRP, aPTT ratio, PT ratio, D-dimers, fibrinogen, FVIII, VWF:Ag and FVIII/VWF:Ag ratio were evaluated.

Statistical analysis

Quantitative variables were expressed as means (standard deviation) in the case of normal distribution or medians (interquartile range, IQR) otherwise. Normality of distributions was assessed using histograms and the Shapiro-Wilk test. Categorical variables were expressed as numbers (percentage). Cumulative incidence of respiratory worsening and thrombotic events within 30 days of admission were estimated using the Kalbfleisch and Prentice method [24] (by taking into account death related to limitation, witholding or withdrawal of life-sustaining treatment as competing event for respiratory worsening, and any death as competing event for thrombotic events). Cumulative incidence of all-cause mortality was estimated using Kaplan-Meier method.

Main biological markers (PaO2/FiO2 ratio, CRP, fibrinogen, D-dimers, FVIII, VWF:Ag, FVIII/VWF:Ag) were compared according to admission type and oxygen requirements at admission by using one-way ANOVA (or Kruskall Wallis test according to normality of distribution); in case of significant difference, post-hoc pairwise comparisons were done using linear contrast (or using Dunn's test).

We assessed the association of biological markers measured at admission to the ED with the occurrence of increase in oxygen requirements, thrombotic events and all-cause mortality within 30-day of admission using univariable and multivariable regression models. For biological markers with a skewed distribution, the log-transformed values were used in regression models. For respiratory worsening and thrombotic events, we used Fine and Gray models before and after pre-specified

adjustment for age, sex, body mass index (BMI), diabetes and hypertension by treating death (related to limitation, witholding or withdrawal of life-sustaining treatment for increased oxygen requirement and all-cause death for thrombotic events) as a competing event. For all-cause mortality, we used Cox's proportional hazard models before and after adjustment for age, sex, BMI, diabetes and hypertension. We assessed the proportional hazard (PH) assumptions by examining the Schoenfeld residuals; in cases of PH departure, the association was modeled using time-dependent coefficients. We assessed the log-linearity assumptions by using restricted cubic spline functions [25]; in cases of departure, the association was modeled using the quartiles of biomarker distributions. Strength of the associations were evaluated by deriving from regression models, the subhazard ratios (Fine-and Gray models) or hazard ratios (Cox model) per one standard deviation increase in biological data as effect sizes (for blood group, effect sizes were calculated for O versus other blood groups).

Multivariate regression analyses were performed after handling missing data on biological markers and covariates using multiple imputation procedure. Imputation procedure was performed using regression-switching approach [26](chained equations with m=10 obtained) under the missing at random assumption using all baseline characteristics (see table 1), with a predictive mean matching method for quantitative variables and logistic regression model (binary, ordinal or multinomial) for categorical variables. Estimates obtained in the different imputed data sets were combined using Rubin's rules[27].

Finally, the association of occurrence of thrombosis events with oxygen requirements and all-cause mortality were investigated using Cox's proportional hazard regression models by treating the occurrence of thrombosis events as a time-dependent covariate; hazard ratio associated with the time period with thrombosis events was derived as effect size.

Statistical testing was performed at the two-tailed α level of 0.05. No correction for multiple testing were done regarding the exploratory nature of the present study and results should be interpreted with caution and as hypothesis-generating. Data were analyzed using the SAS software package, release 9.4 (SAS Institute, Cary, NC).

RESULTS

Clinical and biological characteristics on emergency department admission

Of the 303 patients with high probability or confirmed COVID-19 admitted to the ED at our hospital from March 20th to April 17th, 2020, 243 patients (155 men and 88 women) were included in the study with a median age of 63.9 years. Patients transferred to our hospital from another hospital were not included in the study **(supplemental Figure 1)**. The proportion of patients with hypertension, diabetes and overweight-to-obese (BMI>25) were 48.6%, 23.0% and 76.2% respectively. Other underlying co-morbidities are detailed in **Table 1**. Thirty-two patients were receiving antithrombotic treatment at baseline (DOAC, n=19; VKA, n=8, enoxaparin, n=5).

Upon arrival in the ED, 30.9% of patients were directly admitted to the ICU and 65% were first admitted to the ED. Twenty-three (9.4%) patients were directly discharged home from the ED after medical assessment for standardized ambulatory clinical follow-up. All other patients were hospitalized in the medical ward dedicated to COVID-19.

Median time from illness onset to admission was 8 [IQR, 5-11] days. At admission, 169 (69.5%) patients required oxygen support and all had radiological signs of interstitial pneumonia on chest X-rays or CT-scan. Baseline median respiratory rate and PaO2/FiO2 were 24 [IQR, 20-28] per min and 357 [IQR, 252-448] mmHg respectively. In accordance with local guidelines to prevent overwhelming the virology lab, RT-PCR testing was limited to COVID-19 patients requiring hospitalization. RT-PCR was positive for COVID-19 in 220 (90.5%) patients at baseline.

The values of the main biological markers at baseline are presented in **Table 1**. The PaO2/FiO2 ratio was significantly different according to the admission type with the lowest values for patients directly admitted to the ICU (out-patients, ward and ICU, respectively 505 [461-523], 381 [324-458], 197 [130-302], p<0.001). Also depending on admission type (outpatient, ward or ICU), CRP, D-dimers, fibrinogen and VWF levels were highest for patients directly admitted to the ICU **(Figure 1)**. As RT-PCR testing was in most cases limited to COVID-19 patients requiring hospitalization, respiratory illness at admission was less severe in the RT-PCR negative patient group despite the presence of radiological signs of pneumonia. Among the 23 patients without RT-PCR testing, only 2 were admitted to the medical ward dedicated to COVID-19 whereas the 21 others were outpatients. As shown in **Figure 1**, outpatients had a higher PaO2/FiO2 and a lower respiratory rate (20.6±5 vs 24.4±6, p=0.003) upon admission in ED and none of them needs oxygen supply during follow-up.

As shown in **Figure 2**, CRP, fibrinogen, D-dimers and VWF:Ag levels were increased depending on oxygen requirements at admission, with the lowest levels for patients with no oxygen and the highest for patients requiring high-flow oxygen or invasive ventilation. FVIII levels were not affected by oxygen requirements.

Increase in oxygen requirement and biomarkers

At the time of analysis (May 20th, 2020), all patients either died (n=32) or reached the 30-days follow-up after admission. Respiratory worsening was observed in 71 patients (30-day incidence, 29.2%; 95%CI, 23.6 to 35.0%) during follow-up: 2 patients were re-admitted for dyspnea, 59 required an escalation in oxygen supply (low flow oxygen, n=13; non-invasive ventilation or high flow oxygen devices, n=9;

invasive mechanical ventilation, n=37) and 10 died from acute respiratory distress syndrome (ARDS) without context of limitation, witholding or withdrawal of life-sustaining treatment. In patients presenting an increase in oxygen requirement there was a significant decrease of PaO2/FiO2 ratio the day of increase in oxygen requirement compared to baseline values (172 [122-233] vs 313 [185-396], p<0.01; **supplemental Figure 2**) Most of the increased oxygen requirement events (87.3%, n=62) occurred in the first 10 days following admission **(supplemental Figure 3A)**. Among the 71 patients with aggravation according to the increase of oxygen requirements, we observed no significant difference in the timing of aggravation according to the severity of oxygen requirements (3 [1-4] days for escalation of non-invasive oxygen supply vs 3 [1-5] days for the need of invasive ventilation), however, death from ARDS occurred at a significantly later stage ([12 [5-19] days, p=0.001).

In univariable Fine-and gray regression analysis considering the 16 deaths in the context of limitation of life-sustaining treatment as competing events, increased CRP (SHR, 1.68; 95%CI, 1.26 to 2.23), increased fibrinogen (SHR, 1.32; 95% CI, 1.04 to 1.68) and decreased FVIII/VWF:Ag ratio (SHR, 0.70 ; 95% CI, 0.52-0.96) levels at admission were significantly associated with the risk of respiratory degradation during follow-up **(Table 2).** After adjustment for age, sex, BMI, hypertension and diabetes, these associations were not modified (**Table 2**). In multivariate analysis, the association between decreased lymphocytes and risk of increased oxygen requirement reached the significance level, with an adjusted SHR of 0.71 (95%CI, 0.50 to 0.99). In conclusion, CRP, fibrinogen, the FVIII/VWF ratio and reduced lymphocyte count were all independently associated with the increased need for oxygen support.

Thrombotic events and biomarkers

The cumulative incidence of 30-day any thrombotic event was 12.8% (95% CI, 8.9 to 17.3%); there were 22 patients presenting PE (1 troncular, 3 lobar, 12 segmental and 6 subsegmental), 4 DVT, 1 MI, 2 ischaemic stroke and 2 catheter-related thrombosis of the jugular vein. The 31 thrombotic events occurred with a median time from hospital admission to thrombotic events of 8 [IQR, 1 to 11] days (**supplemental Figure 3B**). As shown in **Table 3**, the proportional subhazard assumptions for several biomarkers were not satisfied with a positive association with occurrence of thrombotic event during the first 5 days for leucocytes (SHR, 1.36; 95%CI, 1.04 to 1.76), platelets (SHR, 1.71; 95%CI, 1.11 to 2.62), D-dimers (SHR, 2.48; 95%CI, 1.66 to 3.78), FVIII (SHR, 1.78; 95%CI, 1.17 to 2.68). Intriguingly a negative association with occurrence of thrombotic event after 5 days was observed for FVIII (SHR, 0.46; 95%CI 0.26 to 0.82) and VIII/VWF:Ag ratio (SHR, 0.47; 95%CI, 0.25 to 0.87). However, patients presenting a thrombotic event after 5 days had lower FVIII levels at baseline compared to patients presenting a thrombotic event before day 5 or no thrombotic event (190±45, 314±143, and 232±82 respectively; p<0.01). After 4 days, FVIII increased in patients presenting a thrombotic event after 5 days and remained stable in patients presenting early thrombosis and the difference between both groups was no longer significant (**supplemental Figure 4**). Time interval between symptom onset and thrombosis was longer in the group with early thrombotic event after admission when compared to late thrombotic event after admission, although not significant (11 [8-12] days versus 9.5 [7-11]).

Thromboembolic complications were significantly associated with a higher risk of increase in oxygen requirements (% per patients-days exposed vs. non exposed to thrombosis events: 1.9% vs. 0.4%), with a HR 2.66 (95%CI, 1.26 to 5.60). Most events occurred in ICU.

We also observed a positive association between thromboembolic events during the entire follow-up period (without deviation to proportional subhazard assumptions) for neutrophils (SHR, 1.40; 95%CI, 1.06 to 1.84), and Lactate dehydrogenase (LDH) (SHR 1.79; 95%CI, 1.36 to 2.36). After adjustment for pre-specified confounders, these associations were not modified (**Table 3**).

All-cause mortality and biomarkers

The 30-day mortality was 13.2% (95%CI, 9.2 to 17.8%) (**supplemental Figure 3C**) with half of patients in a context of limitation, witholding or withdrawal of life-sustaining treatment, mainly limitation of invasive mechanical ventilation in case of worsening respiratory status. Thromboembolic complications were not significantly associated with all-cause mortality (% per patients-days exposed vs. non exposed to thrombosis events: 0.8% vs. 0.4%) with an HR of 2.07 (95%CI, 0.79 to 5.43). However, the numbers of both thrombotic events and deaths were too low to achieve a significant difference. The results are presented in **supplemental Table 4**.

DISCUSSION

In this study, we provide evidence that coagulation biomarkers, including FVIII and VWF, at admission to the ED are associated with the severity of COVID-19 and predict a higher risk of increase in oxygen requirements irrespective of age, sex, BMI, diabetes and hypertension. Our results support the hypothesis that SARS-CoV-2-associated thromboinflammatory hypercoagulability could directly contribute to the underlying pulmonary pathogenesis.

The objective of this study was to assess whether COVID-19 hypercoagulability and especially markers associated with inflammation and endothelial damage such as FVIII and VWF were associated with disease severity. To better understand this role of coagulation in SARS-CoV-2 pneumonia pathogenesis we aimed to adjust the predictive value of coagulation biomarkers to confounding factors such as major comorbidities. Moreover, we aimed to evaluate the severity according to oxygen requirements rather than only admission to ICU or death given that these outcomes associated with COVID-19 are heavily influenced by the presence of other underlying comorbidities.

In our cohort, biomarkers that have been already associated with a poor outcome such as lymphopenia, elevated CRP and fibrinogen were predictive of respiratory worsening[28–30]. Reflecting the inclusion of all consecutive primary admissions irrespective of the initial severity, the initial admitting respiratory rate and PaO2/FiO2 were although not completely normal, within a reasonable spectrum. However, we identified a gradual increase in VWF levels according to oxygen requirements at admission with only 10 patients presenting with normal VWF levels, all of them discharged from the ED a-with outpatient follow-up. Unusually high circulating VWF levels have been reported in patients with severe COVID-19 infection [6,17]. The vascular endothelium is emerging as a key target-organ of SARS-Cov-2. SARS-Cov-2 infects target cells in the lung, heart, intestine and kidney using the angiotensin converting enzyme 2 (ACE2) receptor which is also widely expressed on endothelial cells. Recent autopsy findings suggest that SARS-Cov-2 infection induces widespread endothelial dysfunction and inflammation that could shift the endothelial balance towards a procoagulant state[11].VWF endothelial expression is characterized by a vascular-bed heterogeneity with lung endothelial cells being the first source of circulating VWF [22]. VWF expression and release from endothelial cells Weibel-Palade bodies is also stimulated by hypoxia [33,34]. Hypoxia-induced VWF upregulation is associated with the presence of thrombi in heart and lung vascular beds and promotes recruitment of leukocytes[35].

In our cohort including COVID-19 patients with varying degrees of illness, a decrease in FVIII/VWF ratio values on admission was associated with a higher risk of worsening respiratory status, as evidenced by an increase in oxygen requirements. This suggests that both inflammation and a SARS-CoV-2-induced endotheliopathy could contribute to lung damage pathogenesis. The different levels of FVIII and VWF as evaluated by their ratio could reflect a major synthesis and release of VWF in the lung due to inflammation, hypoxia and direct SARS-CoV-2 destruction of endothelial cells, while the levels of FVIII are less increased because the liver is only exposed to pro-inflammatory cytokines.

Age, male sex, increased BMI and metabolic syndrome are significantly associated with an increased risk of severe forms and death from COVID-19 infection [36] . Importantly, the prediction of an increase in oxygen requirement remained significant after adjusting for these metabolic comorbidities, confirming the direct involvement of procoagulant changes in COVID-19 pneumonia pathogenesis.

Thrombosis was associated with biomarkers reflecting inflammation including increased leucocyte, neutrophil and platelet counts. We also observed an association with higher LDH, D-Dimers and FVIII levels. In a recent meta-analysis, an association between elevated LDH levels measured at earliest time point in hospitalization and worse outcomes was reported in patients with COVID-19 [37] as observed in patients with Middle East Respiratory Syndrome (MERS)[38]. Several mechanisms may account for LDH increase in COVID-19 including thrombotic microangiopathy, upregulation of the glycolytic pathway in a context of severe hypoxia and direct cell damage since this intracellular enzyme is found in pneumocytes, the main target-cell of SARS-CoV-2.

D-dimers at admission or increasing D-dimers over time have been associated with an increased risk of respiratory degradation, thrombosis and death from COVID-19 infection[2,14,39]. Reports to date have not accounted for age or other major comorbidities that contribute to D-dimers elevation as potential cofounders in risk prediction. Of note, in our cohort the predictive value of D-dimers on the occurrence of thrombosis remained significant after adjustment on major metabolic comorbidities associated with COVID-19 and also risk factors for thrombosis such as BMI and age. Similarly, high FVIII levels at admission were also associated with an increased risk of early-onset thrombosis (e.g., in the five days following admission) independently of major comorbidities. Among coagulation factors, high levels of FVIII and VWF were the strongest identified risk factors and recently a causal role of these two proteins in thrombotic events has been suggested [40,41]. As we observed a secondary increase of FVIII in patients with late-onset thrombosis we suspect that this factor can only predict events in a short time frame, and so should be closely and frequently monitored. VWF levels upon admission were not predictive of thrombotic events during follow-up. This could suggest that the important increase in VWF observed in almost all patients related to endothelial injury in the lung, as reflected by the relation with the oxygen requirements at admission is not a marker of patients at higher risk for thrombosis.

Altogether, these biological features make the link between SARS-CoV2 infection and the severity of hypoxemia. Indeed activation of coagulation cascade leading to widespread thrombosis in the lung and endothelial damages are thought to be involved in the disruption of pulmonary vasoregulation especially the vasoconstriction secondary to alveolar hypoxia [42].

The main strengths of our study are: i) our study was prospective with a systematic follow-up in all patients at day-30 ii) in order to prevent referral bias, this study was performed in COVID-19 patients with radiological signs of pneumonia but with varying severity of illness iii) adjustment for established risk factors for disease progression and death and accounting for limitation of life-sustaining treatment as competing events for adverse outcomes are two other major strengths of the present study. iii) potential limitation, witholding or withdrawal of life-sustaining treatment in frail elderly patients is a potential confounder that should be taken into account as a competing event when assessing the association between biomarkers and the risk of adverse outcomes in COVID-19 patients [43]. In our study we considered death with limitation, witholding or withdrawal of life-sustaining treatment as a competing event for the evaluation of respiratory worsening meaning that death with respiratory failure was not considered as worsening when occurring in the context of limited, withheld or withdrawn life-sustaining

treatment. However, this study has three main limitations. First, this was a single-center study, second the thrombotic complications were not diagnosed through systematic screening with doppler ultrasound or CT pulmonary angiogram. The prevalence of VTE and PE was therefore probably underestimated since the access doppler ultrasound and CT pulmonary angiogram was limited in COVID-19 patients hospitalized in ICU for practical reasons. Furthermore, as seasonal Influenza outbreak was over in Europe at the start of the COVID-19 pandemic, we could not include patients with interstitial pneumonia related to other viruses than SARS-Cov2 as controls. It remains to be assessed whether the biomarkers associated with worse outcomes in our study are specific to COVID19 pneumonia or could be translated to pneumonia related to other virus such as SARS, MERS or influenza.

CONCLUSION

We provide evidence that levels of coagulation biomarkers including FVIII and VWF at time of admission to the emergency department are associated with the severity of COVID-19 and predict risk of increased oxygen requirements irrespective of age, sex, body mass index, diabetes and hypertension.

AUTHOR CONTRIBUTIONS

AR and SS designed the study, analyzed the data, and wrote the manuscript. FL, JG, MC, LC, AR, EJ, AD, KF and ML collected clinical data. JL and AD performed the statistical analysis. EK, DG, PL and JP provided critical input and analysis. All authors provided editorial review and assisted in writing the manuscript.

REFERENCES

1. Connors JM, Levy JH. COVID-19 and its implications for thrombosis and anticoagulation. *Blood*. 2020;
2. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J. Thromb. Haemost.* 2020;18(4):844–847.
3. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020;
4. Wu C, Chen X, Cai Y, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med*. 2020;
5. Klok FA, Kruip MJHA, van der Meer NJM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb. Res.* 2020;
6. Helms J, Tacquard C, Severac F, et al. High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. *Intensive Care Med*. 2020;
7. Poissy J, Goutay J, Caplan M, et al. Pulmonary Embolism in COVID-19 Patients: Awareness of an Increased Prevalence. *Circulation*. 2020;
8. Middeldorp S, Coppens M, van Haaps TF, et al. Incidence of venous thromboembolism in hospitalized patients with COVID-19. *J. Thromb. Haemost.* 2020;
9. Wichmann D, Sperhake J-P, Lütgehetmann M, et al. Autopsy Findings and Venous Thromboembolism in Patients With COVID-19. *Ann. Intern. Med.* 2020;
10. Lax SF, Skok K, Zechner P, et al. Pulmonary Arterial Thrombosis in COVID-19 With Fatal Outcome: Results From a Prospective, Single-Center, Clinicopathologic Case Series. *Ann. Intern. Med.* 2020;
11. Varga Z, Flammer AJ, Steiger P, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet*. 2020;395(10234):1417–1418.
12. Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. *N. Engl. J. Med.* 2020;
13. Iba T, Levy JH, Levi M, Connors JM, Thachil J. Coagulopathy of Coronavirus Disease 2019. *Crit. Care Med.* 2020;
14. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497–506.
15. Grasselli G, Zangrillo A, Zanella A, et al. Baseline Characteristics and Outcomes of 1591 Patients Infected With SARS-CoV-2 Admitted to ICUs of the Lombardy Region, Italy. *JAMA*. 2020;
16. Richardson S, Hirsch JS, Narasimhan M, et al. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *JAMA*. 2020;
17. Panigada M, Bottino N, Tagliabue P, et al. Hypercoagulability of COVID-19 patients in Intensive Care Unit. A Report of Thromboelastography Findings and other Parameters of Hemostasis. *J. Thromb. Haemost.* 2020;
18. Escher R, Breakey N, Lämmle B. Severe COVID-19 infection associated with endothelial activation. *Thromb. Res.* 2020;190:62.
19. Pan J, Dinh TT, Rajaraman A, et al. Patterns of expression of factor VIII and von Willebrand factor by endothelial cell subsets in vivo. *Blood*. 2016;128(1):104–109.
20. Liao X, Wang B, Kang Y. Novel coronavirus infection during the 2019-2020 epidemic: preparing intensive care units-the experience in Sichuan Province, China. *Intensive Care Med*. 2020;46(2):357–360.
21. Rocca B, Fox KAA, Ajjan RA, et al. Antithrombotic therapy and body mass: an expert position paper of the ESC Working Group on Thrombosis. *Eur. Heart J.* 2018;39(19):1672–

1686f.
22. Susen S, Tacquard CA, Godon A, et al. Prevention of thrombotic risk in hospitalized patients with COVID-19 and hemostasis monitoring. *Crit Care*. 2020;
23. Société de réanimation de langue. Limitation et arrêt des traitements en réanimation adulte. Actualisation des recommandations de la Société de réanimation de langue française. *Réanimation*. 2010;19(8):679–698.
24. Prentice RL, Kalbfleisch JD, Peterson AV, et al. The analysis of failure times in the presence of competing risks. *Biometrics*. 1978;34(4):541–554.
25. Harrell FE, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med*. 1996;15(4):361–387.
26. Buuren S van, Groothuis-Oudshoorn K. Multivariate Imputation by Chained Equations in R. *J. Stat. Soft.* 2011;45(3):.
27. Gladitz J. Rubin, Donald B.: Multiple Imputation for Nonresponse in Surveys. John Wiley & Sons, Chichester – New York – Brisbane – Toronto – Singapore 1987, xxx, 258 S., 6 Abb., £ 30.25, ISSN 0271-6232. *Biom. J.* 1989;31(1):131–132.
28. Guan W-J, Ni Z-Y, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N. Engl. J. Med.* 2020;382(18):1708–1720.
29. Fogarty H, Townsend L, Ni Cheallaigh C, et al. COVID19 coagulopathy in Caucasian patients. *Br. J. Haematol.* 2020;
30. Ranucci M, Ballotta A, Di Dedda U, et al. The procoagulant pattern of patients with COVID-19 acute respiratory distress syndrome. *J. Thromb. Haemost.* 2020;
31. Nassiri M, Liu J, Kulak S, et al. Repressors NFI and NFY participate in organ-specific regulation of von Willebrand factor promoter activity in transgenic mice. *Arterioscler. Thromb. Vasc. Biol.* 2010;30(7):1423–1429.
32. Shirodkar AV, St Bernard R, Gavryushova A, et al. A mechanistic role for DNA methylation in endothelial cell (EC)-enriched gene expression: relationship with DNA replication timing. *Blood*. 2013;121(17):3531–3540.
33. Mojiri A, Nakhaii-Nejad M, Phan W-L, et al. Hypoxia results in upregulation and de novo activation of von Willebrand factor expression in lung endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* 2013;33(6):1329–1338.
34. Pinsky DJ, Naka Y, Liao H, et al. Hypoxia-induced exocytosis of endothelial cell Weibel-Palade bodies. A mechanism for rapid neutrophil recruitment after cardiac preservation. *J. Clin. Invest.* 1996;97(2):493–500.
35. Mojiri A, Alavi P, Lorenzana Carrillo MA, et al. Endothelial cells of different organs exhibit heterogeneity in von Willebrand factor expression in response to hypoxia. *Atherosclerosis*. 2019;282:1–10.
36. Guo T, Fan Y, Chen M, et al. Cardiovascular Implications of Fatal Outcomes of Patients With Coronavirus Disease 2019 (COVID-19). *JAMA Cardiol*. 2020;
37. Henry BM, de Oliveira MHS, Benoit S, Plebani M, Lippi G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. *Clin. Chem. Lab. Med.* 2020;
38. Assiri A, Al-Tawfiq JA, Al-Rabeeah AA, et al. Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. *Lancet Infect Dis*. 2013;13(9):752–761.
39. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA*. 2020;
40. Rietveld IM, Lijfering WM, le Cessie S, et al. High levels of coagulation factors and venous thrombosis risk: strongest association for factor VIII and von Willebrand factor. *J. Thromb. Haemost.* 2019;17(1):99–109.

41. Sabater-Lleal M, Huffman JE, de Vries PS, et al. Genome-Wide Association Transethnic Meta-Analyses Identifies Novel Associations Regulating Coagulation Factor VIII and von Willebrand Factor Plasma Levels. *Circulation*. 2019;139(5):620–635.
42. Marini JJ, Gattinoni L. Management of COVID-19 Respiratory Distress. *JAMA*. 2020;
43. Vincent J-L, Taccone FS. Understanding pathways to death in patients with COVID-19. *The Lancet Respiratory Medicine*. 2020;8(5):430–432.

FIGURE LEGENDS

Figure 1. Baseline values of main biomarkers according to admission type
Scatter plots of (A) PaO2/FiO2 ratio, (B) CRP, (C) Fibrinogen, (D) D-dimers, (E) FVIII, (F) VWF:Ag and (G) FVIII/VWF:Ag ratio . Bars indicate median and interquartile range or means ±standard deviation as appropriate. ICU=Intensive Care Unit**.**
* p<0.05.

Figure 2. Baseline values of main biomarkers according to oxygen requirements at admission to the emergency department
Scatter plots of (A) CRP, (B) Fibrinogen, (C) D-dimers, (D) FVIII, (E) VWF:Ag and (F) FVIII/VWF:Ag ratio . Bars indicate median and interquartile range or means ±standard deviation as appropriate.
* p<0.05.

Table 1. Patient clinical and biological characteristics at admission to the emergency department in the overall study population

Characteristics	N	Values
Age, years (mean ± SD)	243	63.9 ± 16.2
Male sex	243	155 (63.8)
Body mass index, kg/m^2 (mean ± SD)	200	28.0 ± 6.1
Chronic medical illness		
Diabetes	243	56 (23.0)
Hypertension	243	118 (48.6)
Chronic pulmonary disease	243	38 (15.6)
Cardiopathy	243	36 (14.8)
Myocardial infarction	243	22 (9.1)
Stroke	243	22 (9.1)
Hepatopathy	243	5 (2.1)
Chronic renal failure	243	17 (7.0)
Active cancer	243	23 (9.5)
Immunocompromised	242	11 (4.5)
Number of medical illness	243	
None		79 (32.5)
One		65 (26.8)
More than one medical illness		99 (40.7)
Illness characteristics		
Time from illness onset to admission, days	243	8 (5 to 11)
Admission type		
Emergency department	243	158 (65.0)
Ward		10 (4.1)
ICU		75 (30.9)
Severity of respiratory illness at admission		
Respiratory rate, /min (mean SD)	236	24.0±5.9
PaO2/FiO2 ratio (mmHg)	236	357 (252 to 448)
Oxygen requirement at admission	243	
no oxygen		74 (30,5)
supplemental oxygen		102 (42)
non-invasive ventilation or high flow oxygenation		20 (8,2)
invasive mechanical ventilation		47 (19,3)
Biological data		
ABO blood group	192	
A		85 (44.3)
AB		11 (5.7)
B		19 (9.9)

O		77 (40.1)
Leucocytes,/mm^3(mean ± SD)	229	7674 ± 3749
Neutrophils, /mm^3	181	5000 (3700 to 7000)
Lymphocytes, /mm^3 (mean ± SD)	182	1060 ± 611
Monocytes, /mm^3	182	400 (300 to 700)
Platelets, G/L(mean ± SD)	238	228 ± 113
Creatinine, mg/L	227	8 (7 to 11)
Lactate dehydrogenase, IU/L	199	377 (286 to 479)
Troponin, ng/L	204	15.0 (7.5 to 25.5)
CRP, mg/L	227	69 (31 to 126)
aPTTr	211	1.13 (1.03 to 1.23)
PTr	211	1.10 (1.04 to 1.16)
D-dimers, µg/mL	227	1.00 (0.70 to 1.80)
Fibrinogen, g/L (mean ± SD)	227	6.1 ± 1.6
FVIII, IU/dL (mean ± SD)	210	241 ± 96
VWF:Ag, IU/dL (mean ± SD)	212	361 ± 128
FVIII/VWF:Ag ratio (mean ± SD)	210	0.72 ± 0.27

Values are number (%) or median (interquartile range) unless otherwise as indicated.
Abbreviations: aPTTr= activated partial thromboplastin time ratio; PTr=prothrombin time ratio; SD=standard deviation.

Table 2. Associations of biological data with 30-day increase of oxygenation requirements

	30-day aggravation		Unadjusted		Adjusted[1]	
Biological data	**No (n=172)**	**Yes (=71)**	**SHR (95%CI)**	**P**	**SHR (95%CI)**	**P**
O blood group, n(%)	56 (42.1)	21 (35.6)	0.78 (0.46 to 1.32)[2]	0.35	0.80 (0.46 to 1.39)[2]	0.44
Leucocytes, /mm[3]	7676 ± 3796	7667 ± 3663		0.31[6]		0.26[6]
0 to 5 days[4]			1.12 (0.89 to 1.40)	0.16	1.13 (0.89 to 1.43)	0.32
6 to 30 days[4]			0.65 (0.31 to 1.33)	0.24	0.66 (0.32 to 1.34)	0.24
Neutrophils, /mm[3]	4700 (3600 to 6500)	5350 (4400 to 7500)	1.29 (0.96 to 1.74)[3]	0.090	1.19 (0.88 to 1.61)[3]	0.24
Lymphocytes, /mm[3]	1109 ± 646	927 ± 485	0.74 (0.54 to 1.01)	0.061	0.71 (0.50 to 0.99)	0.041
Monocytes, /mm[3]	500 (300 to 700)	400 (300 to 700)	0.97 (0.75 to 1.25)[3]	0.81	0.91 (0.72 to 1.16)[3]	0.44
Platelets, G/L	236 ± 120	214 ± 98		0.073[6]		0.28[6]
0 to 5 days[4]			0.94 (0.73 to 1.21)	0.62	0.95 (0.72 to 1.24)	0.69
6 to 30 days[4]			0.66 (0.45 to 0.94)	0.023	0.66 (0.43 to 1.01)	0.057
Creatinine, mg/L	8 (7 to 11)	10 (7 to 12)	1.20 (0.95 to 1.51)[3]	0.12	1.10 (0.83 to 1.47)[3]	0.48
Lactate dehydrogenase, IU/L	359 (280 to 476)	426 (312 to 508)	1.22 (0.96 to 1.54)[3]	0.090	1.11 (0.87 to 1.43)[3]	0.38
Troponin[5] ng/L	14 (7 to 26)	16 (9 to 24)		0.25[6]		0.50[6]
<8	41 (28.1)	10 (17.2)	1.00 (ref.)	-	1.00 (ref.)	-
8 to 14	33 (22.6)	17 (29.3)	1.90 (0.88 to 4.04)	0.098	1.16 (0.61 to 2.18)	0.65
15 to 25	34 (23.3)	18 (31.0)	1.99 (0.93 to 4.23)	0.075	1.19 (0.60 to 2.31)	0.62

>25	38 (26.0)	13 (22.5)	1.36 (0.61 to 3.04)	0.46	0.71 (0.33 to 1.53)	0.38
C-reactive protein, mg/L	60 (25 to 111)	101 (56 to 143)	1.68 (1.26 to 2.23)[3]	<0.001	1.66 (1.25 to 2.19)[3]	<0.001
aPTT	1.13 (1.00 to 1.26)	1.13 (1.06 to 1.23)	1.02 (0.85 to 1.23)[3]	0.82	1.02 (0.84 to 1.24)[3]	0.85
PTr	1.09 (1.04 to 1.16)	1.10 (1.05 to 1.18)	1.15 (0.98 to 1.35)[3]	0.081	1.10 (0.89 to 1.38)[3]	0.34
D-dimers, µg/mL	1.00 (0.70 to 1.80)	1.00 (0.80 to 1.90)	1.04 (0.77 to 1.40)[3]	0.80	1.01 (0.74 to 1.38)[3]	0.95
Fibrinogen, g/L	5.9 ± 1.7	6.4 ± 1.5	1.32 (1.04 to 1.68)	0.021	1.34 (1.04 to 1.74)	0.022
Factor VIII, IU/dL	244 ± 104	233 ± 79	0.93 (0.73 to 1.17)	0.51	0.89 (0.71 to 1.13)	0.34
VWF:Ag[5], IU/dL	351 ± 141	381 ± 98		0.30[6]		0.39[6]
<270	44 (28.6)	9 (15.5)	1.00 (ref.)	-	1.00 (ref.)	-
270 to 354	35 (22.7)	17 (29.3)	2.09 (0.94 to 4.60)	0.068	1.71 (0.77 to 3.78)	0.18
355 to 430	38 (24.7)	16 (27.6)	1.86 (0.83 to 4.14)	0.13	1.86 (0.87 to 3.93)	0.11
>430	37 (24.0)	16 (27.6)	1.94 (0.86 to 4.35)	0.11	1.80 (0.82 to 3.97)	0.14
FVIII/VWF:Ag ratio	0.74 ± 0.27	0.64 ± 0.24	0.70 (0.52 to 0.96)	0.025	0.71 (0.51 to 0.98)	0.036

Values are median (interquartile range) or means ±standard deviation. SubHazard ratios (SHRs) were calculated using Fine and Gray models taking into account the mortality in the context of withholding or withdrawal of life-sustaining treatment (n=16) as competing events and were expressed per one standard deviation unless otherwise as indicated.

[1] adjusted for age, sex, body mass index, hypertension and diabetes calculated after handling missing values (in biological and confounding factors) by multiple imputation; [2] SHR calculated for O versus others blood groups; [3]SHR calculated per one standard deviation in log-transformed values.

[4] modeled with time dependent coefficients to accommodate deviation in proportional subhazard assumption.

[5] modeled as categorical variables based on quartiles to accommodate deviation in log linear relationship

[6] p-value for overall effect calculated using a likelihood ratio test.

Abbreviations: aPTTr= activated partial thromboplastin time ratio; CI=confidence interval; PTr= prothrombin time ratio; CRP=C-reactive protein; FVIII: factor VIII activity; SD=standard deviation; SHR=subHazard ratio; VWF:Ag: von Willebrand factor antigen.

Table 3. Associations of biological data with 30-day thrombotic events

	30-day thrombotic Events		Unadjusted		Adjusted[1]	
Biological data	**No (n=212)**	**Yes (=31)**	**SHR (95%CI)**	**P**	**SHR (95%CI)**	**P**
O blood group, n(%)	67 (41.6)	10 (32.3)	0.69 (0.32 to 1.45)[2]	0.32	0.76 (0.35 to 1.61)[2]	0.47
Leucocytes, /mm^3	7542 ± 3784	8512 ± 3461		0.070[5]		0.14[5]
0 to 5 days[4]			1.36 (1.04 to 1.76)	0.022	1.50 (1.11 to 2.03)	0.008
6 to 30 days[4]			1.02 (0.72 to 1.45)	0.89	1.11 (0.74 to 1.65)	0.60
Neutrophils, /mm^3	4900 (3600 to 6500)	6550 (5200 to 8200)	1.40 (1.06 to 1.84)[3]	0.018	1.42 (1.03 to 1.95)[3]	0.031
Lymphocytes, /mm^3	1070 ± 633	974 ± 368	0.85 (0.60 to 1.20)	0.36	0.92 (0.60 to 1.40)	0.69
Monocytes, /mm^3	400 (300 to 700)	400 (200 to 800)		0.086[5]		0.35[5]
0 to 5 days[4]			1.47 (0.79 to 2.73)[3]	0.22	1.28 (0.53 to 3.07)[3]	0.57
6 to 30 days[4]			0.72 (0.51 to 1.02)[3]	0.064	0.75 (0.50 to 1.11)[3]	0.15
Platelets, G/L	226 ± 112	246 ± 120		0.020[5]		0.053[5]
0 to 5 days[4]			1.71 (1.11 to 2.62)	0.014	1.71 (1.10 to 2.66)	0.016
6 to 30 days[4]			0.78 (0.52 to 1.18)	0.23	0.78 (0.51 to 1.18)	0.24
Creatinine, mg/L	8.5 (7 to 11.5)	8.0 (6 to 11)	0.84 (0.58 to 1.21)[3]	0.35	0.92 (0.62 to 1.34)[3]	0.64
Lactate dehydrogenase, IU/L	360 (280 to 456)	495 (383 to 599)	1.79 (1.36 to 2.36)[3]	<0.001	1.87 (1.36 to 2.58)[3]	<0.001
Troponin, ng/L	15.0 (7.5 to 26.0)	11.5 (7.5 to 23.0)	1.08 (0.69 to 1.70)[3]	0.73	1.35 (0.84 to 2.15)[3]	0.21
C-reactive protein, mg/L	69 (31 to 123)	79 (28 to 157)	1.08 (0.71 to 1.62)[3]	0.73	1.20 (0.77 to 1.86)[3]	0.40
aPTTr	1.10 (1.03 to 1.23)	1.17 (1.03 to 1.26)	1.25 (0.89 to 1.73)[3]	0.19	1.10 (0.71 to 1.72)[3]	0.66

PTr	1.09 (1.04 to 1.15)	1.15 (1.08 to 1.20)	1.13 (0.94 to 1.35)[3]	0.17	1.10 (0.83 to 1.46)[3]	0.51
D-dimers, µg/mL	1.00 (0.70 to 1.60)	1.60 (1.10 to 4.20)		<0.001[5]		<0.001[5]
0 to 5 days[4]			2.48 (1.66 to 3.70)[3]	<0.001	2.90 (1.86 to 4.50)[3]	<0.001
6 to 30 days[4]			1.01 (0.31 to 3.30)[3]	0.99	1.21 (0.33 to 4.40)[3]	0.77
Fibrinogen, g/L	6.1 ± 1.6	5.9 ± 1.8	0.90 (0.60 to 1.32)	0.31	0.96 (0.65 to 1.41)	0.84
Factor VIII, IU/dL	239 ± 91	251 ± 123		<0.001[5]		0.007[5]
0 to 5 days[4]			1.78 (1.17 to 2.68)	0.006	1.72 (1.15 to 2.55)	0.007
6 to 30 days[4]			0.46 (0.26 to 0.82)	0.008	0.50 (0.27 to 0.91)	0.022
VWF:Ag, IU/dL	358 ± 131	381 ± 107	1.18 (0.90 to 1.55)	0.22	1.23 (0.92 to 1.63)	0.16
FVIII/VWF:Ag ratio	0.72±0.27	0.67±0.29		0.035[5]		0.063[5]
0 to 5 days[4]			1.23 (0.69 to 2.16)	0.48	1.09 (0.61 to 1.92)	0.77
6 to 30 days[4]			0.47 (0.25 to 0.87)	0.015	0.47 (0.26 to 0.85)	0.012

Values are median (interquartile range) or means ±standard deviation. SubHazard ratios (SHRs) were calculated using Fine and Gray models taking into account the mortality (n=27) as competing events and were expressed per one standard deviation unless otherwise as indicated.

[1] adjusted for age, sex, body mass index, hypertension and diabetes calculated after handling missing values (in biological and confounding factors) by multiple imputation. [2] SHR calculated for O versus others blood groups;[3] SHR calculated per one standard deviation in log-transformed values.

[4] modeled with time dependent coefficients to accommodate deviation in proportional subhazard assumption.

[5] p-value for overall effect calculated using a likelihood ratio test.

Abbreviations: aPTTr= activated partial thromboplastin time ratio; CI=confidence interval; PTr= prothrombin time ratio; CRP=C-reactive protein; FVIII: factor VIII activity; SD=standard deviation; SHR=subHazard ratio; VWF:Ag: von Willebrand factor antigen.